

Bee T



Gel Extraction of DNA (Spin Column Extraction)

NucleoSpin® PCR clean-up Gel Extraction Kit

1. Excise gel slice containing DNA fragment of interest.

- Gel electrophoresis fractionates DNA fragments.
- The gel is exposed to UV to find the DNA fragments (stained by Ethidium bromide).
- The goal DNA band is identified.
- Physically remove the slice of gel contains the goal DNA with clean surgical blade.

2. DNA Purification

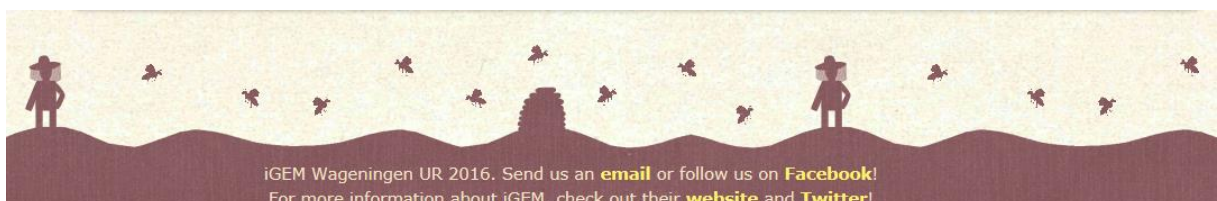
- Determine the appropriate volume of the gel slice by weighing it in a clean 1.5 ml Eppendorf tube.
- Add an 200 μ L Binding Buffer (NT1) per 100mg gel
- Incubate the mixture at 50°C for 5-10 min or until the gel has completely melted.
- Place a NucleoSpin® PCR clean-up Gel column in a provided 2 ml collection tube.
- Apply 700 μ l of the DNA/agarose solution to the NucleoSpin® PCR clean-up Gel column, and centrifuge at 11,000 x g for 30s at room temperature.
- Discard liquid and place the NucleoSpin® PCR clean-up Gel column back into the same collection tube. For volumes greater than 700 μ l, load the column and centrifuge successively, 700 μ l at a time. Each NucleoSpin® PCR clean-up Gel column has a total capacity of 25 μ g DNA. If the expected yield is larger, divide the sample into the appropriate number of columns.
- Add 700 μ l of Buffer NT3 into the NucleoSpin® PCR clean-up Gel column. Centrifuge at 11,000 x g for 30s at room temperature to wash the column. Discard the flow-through and re-use the collection tube.
- Repeat step k with another 700 μ l of Buffer NT3
- Discard liquid and centrifuge the empty NucleoSpin® PCR clean-up Gel column for 1 min at 11,000 x g to dry the column matrix. Do not skip this step, it is critical for the removal of ethanol from the NucleoSpin® PCR clean-up Gel column.
- Place a NucleoSpin® PCR clean-up Gel column into a clean 1.5 ml eppendorf tube. Add 15-30 μ l (depending on desired concentration of final product) of Elution Buffer (10 mM Tris-HCl, pH 8.5) directly onto the column matrix and incubate at room temperature for 1 minute. Centrifuge for 1 min at 11,000 x g to



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elute DNA. This represents approximately 70% of bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration.



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